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# Genetic testing for glucokinase mutations in clinically selected patients with MODY: a worthwhile investment

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## Summary

The differential diagnosis for children with diabetes includes a group of monogenic diabetic disorders known as maturity-onset diabetes of the young (MODY). So far, six underlying gene defects have been identified. The most common subtypes are caused by mutations in the genes encoding the transcription factor HNF-1α (MODY 3) and the glycolytic enzyme glucokinase (GCK) (MODY 2). MODY 2 is the most benign form of diabetes as the threshold for glucose sensing is elevated resulting in mild, regulated hyperglycemia. MODY 2 may usually be treated with diet alone without risk of microvascular complications. Patients with MODY usually present as children or young adults. Genetic testing for MODY in diabetic subjects is often not performed because of the costs and its unavailability in Switzerland. We describe the impact of the genetic analysis for MODY 2 on diabetes management and treatment costs in a five-year-old girl. The patient and her diabetic mother were both found to have a heterozygous missense mutation (V203A) in the glucokinase gene. The five-year-old girl was started on insulin therapy for her diabetes but because her $HbA_{1c}$ remained between 5.8–6.4% (reference 4.1–5.7%) and her clinical presentation suggested MODY insulin was discontinued. She is now well controlled on a carbohydrate controlled diet regimen only. Omission of insulin treatment made regular blood glucose monitoring unnecessary and removed her risk of hypoglycemia. Costs for the genetic analysis were 500 Euro. At our centre costs for diabetes care of a patient with type 1 diabetes are approximately 2050 Euro/year compared to 410 Euro/year for the care of a patient with MODY 2. In addition, a diagnosis of MODY 2 may reassure patients and their families, as microvascular complications are uncommon. Thus there are both health and financial benefits in diagnosing MODY 2. We recommend genetic testing for MODY 2 in clinically selected patients even though this analysis is currently not available in Switzerland and costs are not necessarily covered by the health insurances.

*Key words: MODY; molecular diagnostic; costs; diabetes mellitus*

## Introduction

Not all children with diabetes have type 1 diabetes. One differential diagnosis is maturity-onset diabetes of the young (MODY) which accounts for approximatively 1–2% of diabetes in Europe [1]. MODY is a group of monogenetic forms of diabetes which are characterized by a β-cell dysfunction with an autosomal dominant inheritance and an early onset (<25 y) of diabetes mellitus that is not insulin dependent [2]. To date, six subtypes of MODY have been genetically characterized [3]. Mutations in pancreatic β-cell transcription factor genes cause MODY 1 (hepatocyte nuclear factor (HNF)-4α, OMIM 125850), MODY 3 (HNF-1α, OMIM 600496), MODY 4 (insulin promoter factor-1, OMIM 606392), MODY 5 (HNF-1β, OMIM 604284) and MODY 6 (Neuro-D1, OMIM 606394) and mutations in the gene encoding the glycolytic enzyme glucokinase (GCK) cause MODY 2 (OMIM 125851).

Studies from the UK, France and Italy have shown that MODY 2 and MODY 3 are the most common forms of MODY in children in Europe [1, 4]. MODY 2 and MODY 3 differ fundamen-

**Abbreviations:**
MODY maturity-onset diabetes of the young
NIDDM non-insulin dependant diabetes
GCK glucokinase

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tally in their underlying defect and in their clinical impact, thus necessitating different therapeutic approaches [5–8]. Patients with MODY 2 merely have a higher threshold for insulin secretion from the glucose-sensing β-cell of the pancreas resulting in life long mild regulated hyperglycemia that deteriorates little with age. Thus, patients with MODY 2 rarely suffer from diabetic complications and may be managed by a healthy lifestyle alone as long as they do not develop type 2 diabetes as well [1]. In contrast, patients with MODY 3 have a progressive defect in insulin secretion frequently resulting in severe and progressive hyperglycemia in adult life. Therefore patients with MODY 3 need intensive glycemia treatment, usually with sulphonylureas, to prevent significant long term microvascular complications [1].

Clinical features can help determine which diabetic children have MODY and the likely clinical subtype. MODY patients, in contrast to type 1 do not become severely insulin deficient within 2 years of diagnosis. A detailed family history (including testing of parents) is likely to show autosomal dominant inheritance of diabetes in MODY but this is uncommon in type 1 diabetes. In contrast to type 2 diabetes in children MODY patients are typically lean. Clinical characteristics also help to distinguish between MODY 2 and MODY3. A patient with MODY 2 presents with a fasting blood sugar which is higher than 5.5 mmol/l even at a very young age, but the blood sugar will only increase slightly (typically <3.0 mmol/l) during an oral glucose tolerance test (OGTT) [9]. In contrast, a patient with MODY 3 may have fasting blood glucose values lower than 5.5 mmol/l in early childhood, but these values will increase with age, and at any age blood sugar usually increases by more than 3.0 mmol/l at 2 hours during an OGTT [9]. The differences in OGTT cannot be used for diagnosis as they are not sufficiently sensitive or specific especially below the age of 10 years [9]. To make a diagnosis of the specific type of MODY genetic testing is required. However genetic testing for monogenetic forms of diabetes has been considered controversial because of its high costs. Routine genetic testing for MODY 2 is currently not available in Switzerland and costs for genetic testing are not routinely covered by health care providers.

Here we present a five-year-old girl and her family in whom genetic testing revealed a glucokinase mutation confirming MODY 2. Costs of genetic testing are weighed against intensive diabetes control management, which is necessary for all forms of diabetes apart from MODY 2.

## Case presentation

A 5-year-old Italian girl was noted to be more tired, lethargic, and pale. As her diabetic mother suffered from similar symptoms when her diabetes was first diagnosed at age 25, she measured several random blood glucose levels on her daughter which were between 6.9 and 8.2 mmol/l. At her Primary Care Physician's office, the girl's $HbA_{1c}$ was 7% but spot urine analysis revealed neither glucose nor ketones. The girl was referred to our centre for further evaluation and treatment.

At her first visit we found a healthy, 4 3/12 year old girl. Her weight was 18.8 kg (P90), height was 107 cm (P75-90), and her complete physical exam was normal. Her random blood glucose was 8.9 mmol/l, $HbA_{1c}$ was 6.8% (reference 4.1–5.7%). Past medical history revealed a normal pregnancy except for slightly elevated maternal blood glucose levels, which were controlled by diet alone; her birth weight at term was 2900 g (P10). Postnatal growth and development were normal. The patient's family history revealed at least five family members over three generations who were diagnosed with mild diabetes (figure 1). The available clinical data of these affected family members are summarized in table 1.

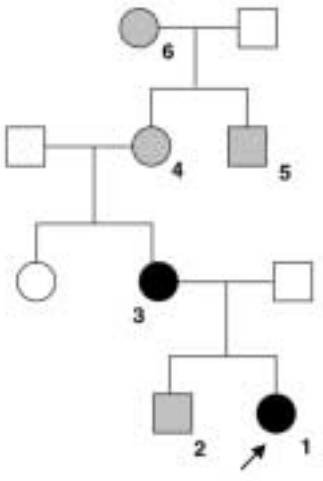


**Figure 1**
Family tree with unaffected subjects shown in white, clinically affected subjects shown in grey, and subjects genetically diagnosed with MODY 2 shown in black. The arrow points to the index patient.

For further evaluation an oral glucose tolerance test (OGTT) was performed [10]. During the OGTT her blood glucose increased from 6.8 mmol/l to 11.8 mmol/l at 30 min but declined to 8.1 mmol/l at 120 min consistent with the diagnosis of impaired glucose tolerance. However, repeated fasting and random glucose levels on occasions exceeded 7.0 mmol/l and 11.2 mmol/l respectively, consistent with the diagnosis of diabetes mellitus according to the WHO criteria. Serum C- peptide and insulin levels were normal. Repeat HbA1c was 6.6% (reference 4.1–5.7%), fructosamine was 265 µmol/l (reference 205–285 µmol/l), and antibodies (anti-GAD, -IA2, -insulin and -β-cell) were all negative.

The initial differential diagnosis was the early presentation of type 1 diabetes [11] or MODY. Therefore, the patient was hospitalised for one week, started on a qualitative and quantitative diet, and a trial of daily subcutaneous insulin injections (4 units glargine, Lantus®, Aventis Pharma; (0.2 U/kg/d)) controlled by blood glucose monitoring (4–5 times per day). However, follow-up visits in our outpatient clinic three and six months later revealed an unchanged HbA1c under this treatment. Also, insulin treatment and the fixed diet regimen made the daily life of the patient and her family very stressful as the five-year-old protested vigorously against the subcutaneous insulin injections and often refused to eat the planned meals, which put her at risk for hypoglycemia on insulin treatment. Thus insulin treatment was discontinued.

**Table 1**
Clinical characteristics of the patients.

| Pt | Patient (age in years) | Age at dx | Symptoms at diagnosis (dx) | Treatment | A1c | BMI (kg/m$^2$) | Complications | Other data |
|---|---|---|---|---|---|---|---|---|
| 1 | Index pt (5) | 5 years | tiredness | Diet, insulin (glargine) and repaglinide | 6.1% | 16.6 | None | 2900 g at birth |
| 2 | Brother (4) | 4 years | none | Diet | 6.4% | | None | 4800 g at birth |
| 3 | Mother (35) | 25 years | tiredness | Diet, glibornurid and metformin | 6.0% | 30 | None | 2 normal pregnancies |
| 4 | Maternal Grandmother (61) | 40ies | thirst, tiredness | Diet, metformin and insulin | 6.5–7% | 31 | None | 2 normal pregancies |
| 5 | Maternal Great-Uncle (65) | 50ies | none | Diet and oral drugs | ? | obese | None | |
| 6 | Great-Grandmother (?) | 60ies | ? | Diet and oral drugs (was on insulin for 1 year) | ? | ? | Unknown | Died of pneumonia |

Based on the clinical presentation with elevated fasting glucose values, mild increase of blood sugars during OGTT, mildly elevated HbA1c, as well as the lack of response to insulin treatment and the typical family history of long standing diabetes without complications in several family members, we recommended genetic testing for MODY 2.

## Methods

Blood was obtained from the patient and her diabetic mother and genomic DNA was extracted from peripheral leukocytes (QIAamp®, Qiagen). Exons 1–10 of the glucokinase gene on chromosome 7p15-p13 were PCR-amplified and purified as previously described [12]. PCR products were sequenced in both directions using the BigDye Terminator Cycle sequencing chemistry (Applied Biosystems) according to the manufacturer's instructions. Reactions were then run on an ABI Prism 3100 DNA Sequencer (Applied Biosystems) and analysed using Sequence Analysis (Applied Biosystems) and Staden Mutation Analysis software.

## Results and follow-up

Genetic analysis revealed that our patient was heterozygous for the missense mutation GTG>GCG in exon 6 of the glucokinase gene resulting in the substitution of alanine for valine at codon 203 (V203A) (figure 2). The patient's mother was heterozygous for the same mutation confirming the autosomal dominant trait. This mutation has been previously described [5, 13]. Recently reported functional studies of the mutant V203A GCK protein have confirmed that this is an aetiological mutation as it markedly alters both the $IC_{50}$ and $V_{max}$ of the enzyme [14–16].

The diagnosis of MODY 2 in our patient confirmed our clinical decision that this diabetic child may be managed by diet alone. Follow-up studies of MODY 2 patients have shown no or little complications [1]. Thus insulin treatment was stopped. Three months after discontinuing insulin our patient presented healthy, her fasting glucose was 7.1 mmol/l and her $HbA_{1c}$ was 6.3%. At this time we also drew fasting glucose and $HbA_{1c}$ on her four-year-old brother (5.7 mmol/l and 6.4%) and on her father (4.4 mmol/l and 5.2%). While glucose and $HbA_{1c}$ were normal in the father, the presence of a fasting glucose >5.5 mmol/l in the patient's brother is consistent with the diagnosis of MODY 2. Thus we recommended including the younger brother in the healthy eating diet regimen at home. Overall the family was very happy about the changes in the daily management of the diabetes, not having to give their child daily insulin injections any longer and measuring glucose only once a week and during illnesses. Furthermore, it was also an enormous psychological relief to the parents that after discontinuation of the insulin treatment they did not have to worry about hypoglycemia any longer. In addition, with respect to the future of their child diagnosis of MODY 2 was a big relief as it is rarely associated with complications.

As the costs for genetic analysis for MODY 2 are currently not routinely covered by the health insurances in Switzerland, we evaluated the cost effectiveness of the genetic testing in our patient. We calculated the costs for testing and the costs for intensive diabetes control and treatment management as recommended for type 1 diabetes. The genetic analysis for MODY 2 (and MODY 3) is currently offered for 500 Euro for the index patient and for 120 Euro for relatives at the Molecular Genetics Department of the Royal Devon & Exeter NHS Foundation Trust (Exeter, UK) (http://www.diabetesgenes.org). Initially, routine diabetes control in our patient – as recommended

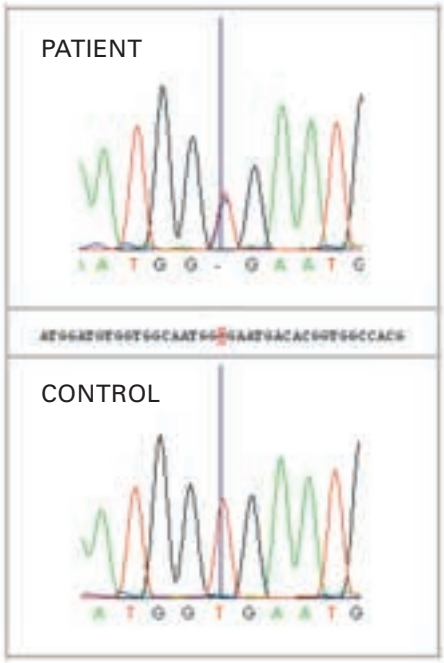


**Figure 2**
Sequence analysis showing the heterozygous GTG>GCG mutation in the patient but not the control. This mutation causes the V203A mutation in the glucokinase.

for type 1 diabetic children-included daily blood glucose monitoring (1150 Euro/year), insulin injections (300 Euro/year), and follow-up visits at our diabetes clinic four times per year (600 Euro/year). Because diabetic children under insulin treatment have a high risk for hypoglycemia, emergency treatment for hypoglycemia may even cause additional costs. Not knowing the underlying defect of MODY diabetes in our patient, we would have recommended follow up visits 2 to 4 times per year for 300–600 Euro/year whereas after genetic diagnosis of MODY 2 we feel safe seeing her once per year. In summary, management changes after genetic testing for MODY 2 in our patient outweigh the costs for the genetic analysis in less than 2 years.

## Discussion

MODY is a rare cause of diabetes mellitus in the general population. However, in a lean, young child of Caucasian ancestry presenting with mild or moderate hyperglycemia and variably reduced insulin secretion, it might be the most likely diagnosis after excluding latent type 1 diabetes mellitus [2, 17]. Although it is still expensive and thus controversial to diagnose MODY 2 by diagnostic genetic analysis routinely, we show here that genetic testing in clinically selected patients is in fact cost effective as well as having major clinical benefit.

MODY 2 is considered benign when comparing the risk for possible long term complications with all other forms of diabetes which means that pharmacological treatment is rarely needed in contrast to all other forms of diabetes. For MODY 2 follow-up visits may be recommended yearly, and patients are typically well controlled by diet alone. In contrast, patients with MODY 3 should be monitored more often as their glucose homeostasis worsens over time requiring treatment with sulfonylurea at some point [1, 7, 8]. Thus, clinical diagnosis of MODY without knowing the underlying defect specifically will not provide enough information to avoid under-treating or over-treating patients with MODY 3 or MODY 2 respectively.

Moreover, for female patients with MODY 2, it may be critical to know the exact diagnosis during pregnancy. Hattersley et al. [18] have shown that children with mutations in the GCK gene were born with low birth weights when the mother was unaffected. In contrast, affected, untreated mothers had affected off springs of normal birth weight, and finally affected mothers carrying unaffected fetus gave birth to overweight babies. Thus, insulin secretion from the pancreatic β-cell in the fetus is regulated by the maternal blood glucose levels and the fetal mutation status [18]. Therefore, if a pregnant woman with mild gestational diabetes due to MODY 2 is treated with insulin to achieve euglycemia, and the fetus carries the GCK mutation (a 50% chance due to the autosomal dominant inheritance), the fetus might suffer from intrauterine growth retardation (IUGR) [19]. Insulin-dependent fetal growth might be impaired under euglycemic conditions because the fetus carrying a GCK mutation needs higher maternal blood glucose concentrations for stimulating insulin secretion from his β-cell.

In conclusion, we are convinced that in carefully selected diabetic patients – such as young children presenting with fasting hyperglycemia, but only moderate increase of blood sugar during OGTT, and a positive family history – genetic testing for MODY 2 is a vital clinical tool as well as being financially beneficial. We hope that routine testing for those selected patients will become available in Switzerland in the near future. Meanwhile this report may also help to convince Swiss health insurance companies that covering the costs for the molecular analysis for patients with a clinical picture that is highly suggestive for MODY 2 is a worthwhile investment.

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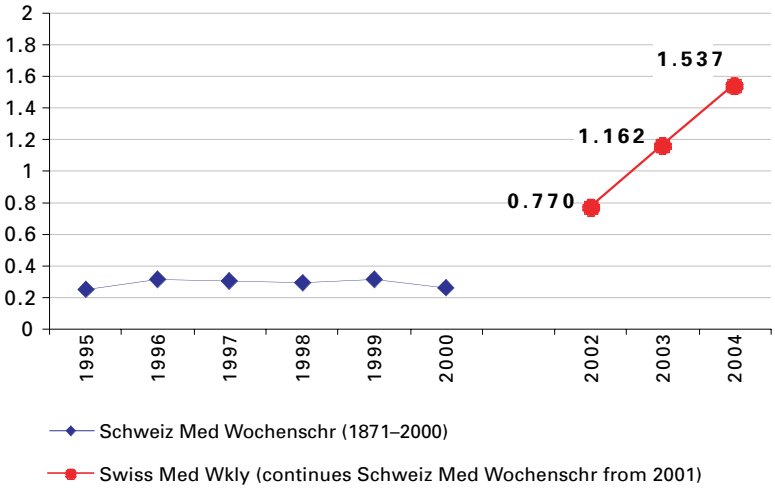


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